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The refraction of light in plant tissues*

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(WITH FOUR TEXT FIGURES)

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I. INTRODUCTION

The laws of refraction have an interest in microscopy, not merely in connection with the definition of anatomical details, but also in the determination of the chemical or physical qualities of any part or product of the cell. Such laws are of universal application, have the advantage of great precision, and serve to differentiate many characters not readily brought out by stains or chemical reagents. Furthermore, a knowledge of the refractive properties of the various tissues is indispensable if one is to obtain the clearest definition of tissue outlines by aid of the microscope. Current methods, however, go little further than listing the indices of refraction of media in which tissues may be mounted, all of which have little significance in anatomical work unless the index of refraction of the tissue substances is also given. In as much as many, if not most, of the ultra-microscopic characters of cell membranes are to a greater or less extent associated with the refractive powers of these membranes, the laws of refraction have their special application in the investigation of the physical or chemical composition as well as in the anatomical characters of cell membranes. Such laws, therefore, in their application to plant tissues, come well within the scope of botanical research, and, as such, open a wide field for investigation, particularly when more than ordinary precision is required.

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* Contribution from the Osborn Botanical Laboratory.

Owing, apparently, to chemical change, the refraction of a plant membrane is peculiarly liable to actual variation during growth; still more characteristic of the plant membrane, due to its hygroscopic qualities, are the apparent fluctuations (deviations) in refraction by virtue of which it absorbs (imbibes) water and other refractive liquids in which it may be immersed.

A study of the refraction of light in plant tissues and of some of the conditions affecting or apparently affecting its stability was carried on by the writer at intervals during the years 1917–1919, at the Osborn Botanical Laboratory. The writer expresses indebtedness to Professor A. W. Evans and other members of the Department of Botany and to the members of the School of Forestry, for every encouragement throughout the investigation; also, to Professor C. S. Hastings of the Department of Physics for many valuable suggestions, and for verification of the work with refractive liquids. The methods described constitute a supplement to the more general technique for woody tissues given in an earlier paper ('19, p. 138).

II. METHODS OF MEASURING REFRACTION WITH THE MICROSCOPE

The methods by which it is possible to determine the refraction of an object (under the microscope) depend on the use of mounting media of known refractive powers. With a gradient series of such media, the index of refraction of an object may be measured with accuracy to the second decimal place.

1. REFRACTIVE LIQUIDS

To measure the refraction of an object microscopically a gradient series of refractive liquids must first be prepared. For plant tissues in the dry condition, a series of twenty liquids differing from each other in refraction by about .005 and ranging from 1.499 to 1.598 was found generally satisfactory. Either aqueous solutions or oils may be used in making up such a series, both water and oil possessing certain advantages over the alternative solvent. Aqueous solutions, for instance, would probably be best for measuring the refractive powers of the living contents of a cell. But such solutions are absorbed by many tissues more than are oils. One in particular of the solutions, to be described presently, the McLean solution, causes great swelling of mucilaginous membranes, which, for certain purposes, is desirable; but unfortunately, it breaks down the structure of starch grains. Water solutions are open to the objection that they tend to evaporate, with the result that the concentration and refraction of the liquids are changed. For general purposes, therefore, non-volatile oil mixtures proved to be superior to aqueous solutions, and a series made up from mixtures of castor oil ($n = 1.49$) and clove oil ($n = 1.535$) for refractions below 1.535, and of clove oil and naphthalene α monobromated ($n = 1.65$) for the higher refractions gave excellent results (n indicating index of refraction). The mixtures and their respective indices of refraction are indicated in TABLE I.

A gradient series of refractive liquids may be prepared by mixing a liquid of high refraction with one of low refraction. When castor oil is mixed with clove oil, or clove oil with naphthalene α monobromated, the index of refraction of the mixture is proportional to the volumes of the liquids mixed. Or, if V_a and V_b are the volumes of the liquids mixed and n_a and n_b the respective indices of refraction, then

$$\frac{V_a(n_a - 1) + V_b(n_b - 1)}{V_a + V_b} = n - 1,$$

If a series of mixtures is now prepared, in each of which mixtures the relative volume of one of the oils of known refraction is decreased by a constant quantity (e.g., 10, 9, 8, . . . 0) while the

TABLE I

A SERIES OF REFRACTIVE OILS USED IN THE DETERMINATION OF RELATIVE REFRACTION

The test liquids are made from mixtures of naphthalene α monobromated (naphth.) clove oil (clove), and castor oil (cast.), and are arranged in sequence according to refraction. O indicates in what media the outlines are invisible or nearly so; + or —, that the refraction is either more or less than that of the liquid.

Test liquids			Lignified and furfurol-reacting tissues							Pectin, cellulose tissues					Cell contents			
Vol.		Index of refraction	Pith, *Tilia*	Vessels, *Tilia*	Normal fibers, *Alphitonia* (secondary wall)	Muci-laginous fibers, *Alphi-tonia*		Bast fibers, *Tilia* (secondary wall)	Middle lamella, *Tilia* (primary wall)	Cambium, middle lamella, *Tilia*	Phloem (soft bast), *Tilia*	Collenchyma *Tilia* = *R.* membrane, *Pyrus*	Primary cortex, *Tilia*	Reserve cellulose, date seed	Starch, wheat	Aleurone, *Zea*	Calcium oxalate trihydrate	Cork, cuticle
Naphth.	Clove					Secondary layer	Mucilaginous layer											
11	9	1.598	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
10	10	1.592	—	—	—	—	—	—	O	—	—	—	—	—	—	—	—	—
9	11	1.587	—	—	—	—	—	—	O	—	—	—	—	—	—	—	—	—
8	12	1.581	—	—	—	—	—	—	+	—	—	—	—	—	—	O	—	—
7	13	1.575	—	—	—	—	—	—	+	—	—	—	—	—	—	O	—	—
6	14	1.569	—	—	—	—	—	—	+	—	—	—	—	—	—	O	—	—
5	15	1.564	—	—	—	—	—	—	+	—	—	—	—	—	—	O	—	—
4	16	1.558	—	—	—	—	O	—	+	—	—	O	—	—	—	O	—	—
3	17	1.552	—	O	O	—	O	—	+	—	—	O	O	—	—	+	+	—
2	18	1.545	—	O	O	—	O	—	+	O	O	O	O	—	—	+	+	—
1	19	1.540	—	O	O	—	O	—	+	O	O	O	O	O	O	+	+	—
0	10	1.535	O	O	O	O	O	—	+	O	O	O	O	O	O	+	+	—
Cast.																		
1	9	1.531	O	O	+	O	O	—	+	O	O	O	O	O	O	+	+	O
2	8	1.526	O	O	+	O	O	—	+	+	O	O	O	O	O	+	+	O
3	7	1.521	O	+	+	+	O	O	+	+	+	O	O	+	O	+	+	O
4	6	1.517	+	+	+	+	O	O	+	+	+	O	O	+	O	+	+	+
5	5	1.512	+	+	+	+	O	O	+	+	+	O	O	+	+	+	+	+
6	4	1.508	+	+	+	+	O	+	+	+	+	O	O	+	+	+	+	+
7	3	1.503	+	+	+	+	+	+	+	+	+	O	O	+	+	+	+	+
8	2	1.499	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

second is correspondingly increased (0, 1, 2, . . . 10), or in such a way that the ratio of volumes of the two liquids (in a series of eleven mixtures) would be to each other in the successive mixtures as $\frac{10}{0}$ $\frac{9}{1}$ $\frac{8}{2}$ $\frac{7}{3}$ . . . $\frac{0}{10}$, then the indices of refraction of the respective mixtures will form a gradient series (see TABLE I). In order to obtain any required difference in refraction between consecutive liquids in the series, it is only necessary to determine the constant by which the relative volumes are consecutively changed.

To measure the volumes, a medicine dropper may be used, but allowance must be made for the relative size of a drop in each case. This may be done by measuring at room temperature (70° F.) the volume of 200 drops of each of the oils used in the mixture. By this means a drop of castor oil was found to have a volume equal to that of 1.8 drops of clove oil, and 1 drop of naphthalene α monobromated, 0.95 the volume of a drop of clove oil.

2. PREPARATION OF THE TISSUES FOR STUDY

In preparing tissues for the measurment of their refraction the following schedule was adopted.

1. Cut as many sections, 10–40 μ thick, as there are refractive liquids in the series, and wash in water for 10 minutes or longer.
2. Transfer each section to a separate slide and cover with a glass slip.
3. Dry for at least fifteen minutes at 100° C. An electric oven is useful for this purpose.
4. Remove the slides one at a time from the oven and mount each section in a different refractive liquid of the series. The samples of tissue are then ready for microscopic examination.

3. DETERMINATION OF RELATIVE REFRACTION

For this purpose, an apochromatic microscope with Abbé condenser and movable sub-stage diaphragm was used, but any microscope with the usual accessories will answer for ordinary purposes. A magnification of about 800 diameters was usually used in the examination. The refraction of the object relative to that of the liquid was determined in each case by one or more of the following methods.

A. By oblique illumination

The partly-closed sub-stage diaphragm is shifted to one side. If the refraction of the tissue mass is above that of the liquid, the outlines appear dark on the side toward which the diaphragm is shifted. But if the refraction of the tissue mass is below that of the liquid, the outlines appear dark on the side away from which the diaphragm is shifted. In TABLE I the refraction of the tissue or body examined is indicated as + (above) or – (below) with respect to the refractive index of the liquid in which it is immersed.

If the dispersion (i.e., the difference between the indices of refraction of light of long and of short wave-length) of the liquid is not the same as that of the object, it is possible to find a common index of refraction for light of one wave-length only. In this case, the refracted rays give fringes of color such as blue on one side and red on the other. McLean's solution, which has a high dispersion, causes wide color fringes with the reserve cellulose of date seed, in dilutions having a refraction between 1.531 and 1.535. This method is particularly useful in determining whether the refraction of an object is above or below that of the liquid.

B. By direct illumination

Relative brightness.—In studying tissues, the relative refraction of a transparent object is often indicated by the illumination of its transverse surface. On raising the tube of the microscope slightly from sharp focus, an object whose refractive index is higher than that of the tissues or liquid in contact with it appears relatively bright except at the border (see FIGS. 1 and 2). An object of lower refraction, on the other hand, appears relatively dark. On lowering the tube the relative brightness is reversed in each case.

The Becke line.—A few bodies, such as the crystals of calcium oxalate in the crystal-parenchyma of woody tissues, are visible in all liquids of the series. The refraction of such bodies is best determined as follows: An uncovered edge of the crystal is found, which projects into the liquid. When brought into sharp focus, a narrow band of bright light—the Becke line—defines the outline. On focusing upward, this band of light appears to move in the direction of the body of highest refraction, i.e., outward from the border into the liquid, if the refraction of the liquid is higher than that of the crystal, or inward from the border toward the centre of the crystal if the refraction of the crystal is higher than that of the liquid.

C. Modifications with hygroscopic tissues

In applying the methods above described to plant tissues, certain modifications are necessary, due to the fact that most membranes tend to imbibe the refractive liquids in which they are immersed and to become invisible in two or more liquids of the series. This latter phenomenon is attributable to the fact that the refraction index of the mass, i.e., the dry tissue substance together with whatever of the refractive liquid may have been imbibed, here approaches that of the liquid in which it is mounted; and under these conditions it would be recorded as O in the tabulation (see TABLE I). In such cases the refractive power of the tissue substance is taken as the mean between the liquid of highest refraction and the one of lowest refraction in which the outlines are barely distinguishable. Further observations regarding the refraction of hygroscopic bodies are made below.

D. Relative accuracy of the methods

The accuracy with which the index of refraction of an object may be determined by the foregoing methods obviously depends, in the first place, upon the accuracy of the calculated indices of the liquid mixtures. In a test made of one of the liquid mixtures (castor oil, 5 volumes: clove oil, 5 volumes), for example, all the figures in the calculated index (1.512 +) were found to be correct; in fact, no errors were found in the calculated indices of any of the mixtures; but for extreme accuracy, the index of refraction of the liquid should be standardized as a matter of precaution.

With accurately standardized liquids, the index of refraction of minerals may, according to Wright ('11, p. 96), be determined with an exactness of ±.001 when monochromatic illumination (strong sodium light) is used, and Wright considers the method by oblique illumination about as accurate as the Becke line method. By the Becke line method the refraction of calcium oxalate trihydrate was found by the writer to lie between 1.568 and 1.552; and with a series of liquids intermediate in refraction between the ones used, it would doubtless have been possible to have made a more exact determination. The method employed to determine the refraction of hygroscopic bodies is less exact than methods applicable to crystalline bodies, but determinations may be carried to the hundredths with reasonable accuracy.

III. REFRACTION OF TISSUES

The results recorded in TABLE I bring out wide relative differences in refraction in different tissues or in different parts of the same tissue; also, it is seen that the index of refraction of any given membrane may deviate widely from the average or may actually change during growth.

1. RELATIVE REFRACTION

As shown in TABLE I, there is no single index of refraction which is common to even the different membranes of a tissue. A fair average for dehydrated tissues would be 1.53, or .01 above that of Canada balsam or dammar (1.52); but the refraction of many bodies, particularly the cell contents, differs widely from the mean. For the dehydrated tissues examined by the writer, *n* (the index of refraction) varies from 1.50 to 1.59; but had protoplasm and other living bodies with high water content been included, far lower refractions would doubtless have been found, and the range in refraction would probably have been about three times as great.

2. DEVIATION IN REFRACTION WITH IMBIBITION

Certain crystalline bodies found in plant tissues, such as the crystals of calcium oxalate, do not become invisible in any of the refractive liquids: no physical change takes place when they are brought in contact with the oil mixtures and no deviation or change in refraction occurs. An organic membrane on the other hand, as already noted, imbibes the liquid in which it is mounted. As a result of the physical change which thus results in the mass of the membrane, the latter becomes invisible, or nearly so, in two or more consecutive liquids of the refractive series, namely, in those whose index of refraction approaches most closely that of the membrane after it has absorbed as much as it will of the refractive liquid in which it is mounted. In other words, the refraction of the membrane mass, after imbibition, deviates from that of the tissue substance according to the volume and refractive index of liquid taken up, the principle involved being nearly the same as that observed in preparing the series of refractive liquids.

Different membranes show great differences in the relative deviation of refraction. Cuticle, cork, true wood fibers, and middle lamella, for example, show relatively little deviation; collenchyma, primary cortex, and mucilaginous membranes, on the other hand, take up relatively more of the liquid and show a correspondingly wide deviation in refraction.

The imbibition of liquid is also indicated by the swelling of the membrane, and those membranes which have the widest deviation in refraction, as, for example, the mucilaginous membranes in woody fibers, are the ones which swell the most. Not infrequently such membranes, as shown by an accurate projection published in a previous paper ('19, p. 143), may swell to almost double thickness when dehydrated sections are mounted in water, glycerine, or McLean's solution. But refraction is a more delicate index than swelling in that it serves to differentiate the particular portions of a membrane which take up the liquid.

Some extremely hygroscopic membranes are invisible, or nearly so, in liquids differing in refraction from one another by more than .055. There thus is an apparently close relation between imbibition and refraction. But since visibility of outline is partly dependent upon other factors, such as the relative light dispersion of the liquid and the object, the deviation in refraction may not be exactly proportional to the imbibition of liquid.

3. CHANGE IN REFRACTION WITH GROWTH

Deviations or apparent fluctuations in refraction, such as those above described, are not associated with any actual change in refraction of the membrane substance; on removing the imbibition liquid from the tissue, the refraction of the membrane is found unchanged. But, during growth, actual changes in refraction may occur, as, for example, in the middle lamella of wood cells. In the early stages of cambial growth, such as may be found in the stems of woody angiosperms, the membrane which subsequently becomes the middle lamella has a refractive index of about 1.53, or the average for plant tissues. In subsequent stages, such as occur in the narrow zone of undifferentiated tissue next to the true cambium, where the thin-walled immature cells are in various stages of elongation, the refraction of this membrane increases with progressive differentiation. Thus, as soon as growth in length of the wood cells has ceased and the walls begin to thicken prominently by apposition—the stage in which the first secondary thickenings of the fibers appear and the tissue hardens—there is a rapid rise in the index of refraction of the middle lamella to 1.59, or .135 above that of glycerine (FIG. 1), at which it remains nearly constant. Such a change in refraction can hardly be explained except in relation to a change in the chemical composition of the pectin lamella, as a result of which, beginning as a membrane of average refraction, it quickly becomes the most refractive of all.

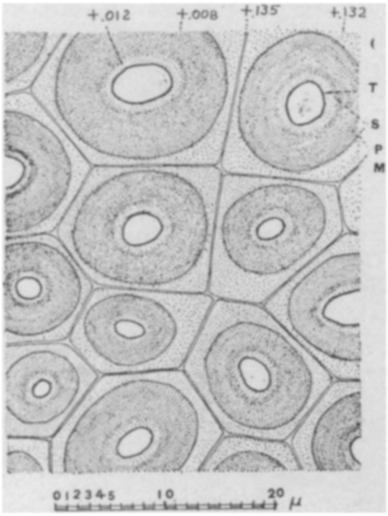

FIG. 1. Abbé camera drawing of fibers of Ipê tabaco (*Tecoma* sp.), mounted in glycerine (n = 1.46). All membranes are + with reference to the glycerine medium. In upper sharp focus, the refractive membranes appear bright; this is indicated in the shading. The middle lamella (*M*) is less distinct in glycerine than indicated in the drawing. The membranes (*M*, middle lamella, and *P*, primary thickening of the fiber) are highly refractive (n = 1.59) and appear brightly illuminated, especially the middle lamella. The secondary layer (*S*) is less refractive (n = 1.54) and relatively dark. *T*, tertiary thickening.

The primary thickening of wood fibers (*P*, FIG. 1) is also composed of a highly refractive material. In xerophytic species of *Tecoma* and a number of other woods, this membrane first appears as a collenchyma-like thickening upon the sides of the middle lamella, in cells which lie near the true cambium, and which have not ceased to elongate and to glide upon one another. This membrane, though much thickened in species of *Tecoma* and certain other genera, is ordinarily thin and, because of similarity in the refractive and staining qualities, is likely to be mistaken as part of a somewhat thickened middle lamella. Both membranes stain deeply with haematoxylin, methylene blue, fuchsin, and other stains for pectic bodies, and together constitute by far the most refractive (.132 –.135 above glycerine) membranes in the mature xylem. But the refraction of the primary wall is slightly lower (about .003) than that of the middle lamella, and, when observed in a refractive medium at which the former becomes invisible (about 1.545 for

Tecoma), the true middle lamella is nearly as sharply defined as in collenchyma (C, FIG. 2).

The observed differences in refraction here are probably associated with differences in mineral content of the membrane studied. Of the mineral elements which enter into the composition of woody tissues, calcium, especially in the form of calcium carbonate, would seem to be the only one likely to bring about an increase in refraction as great as that noted in the middle lamella and primary wall. The presence of silica, for example, in portions of the secondary layer (FIG. 1, *S*) is associated with a much lower refractive index.* The rise in refraction of the middle lamella and primary wall during development finds its most reasonable explanation in the supposition that in these pectic membranes the relative proportion of calcium is greatly increased as soon as the cells have attained their mature size and the mechanical tissues begin to acquire hardness and strength. Apparently, these membranes acquire their mechanical rigidity as a result, in part at least, of the addition of calcium, possibly in combined form, to the pectin; and it is of interest to add that both by chemical treatment and by the action of certain enzymes, as will be described presently, the primary wall may be softened and the middle lamella reduced to a jelly-like consistency, so that cells glide upon one another as during development; even closing membranes of

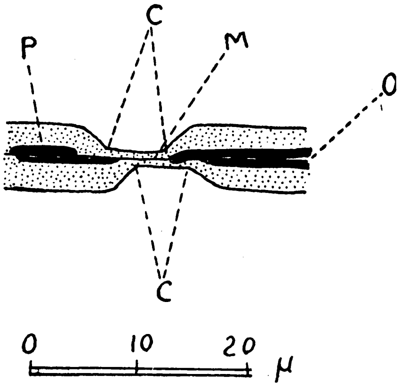

FIG. 2. Longitudinal section of a wall between two thick-walled parenchyma cells in the stem of *Dracaena aurea*, after twenty-four hours treatment in Easter lily pollen enzyme solution. The middle lamella (*M*) has softened, allowing the cells to glide upon another, the closing membrane (*C*) of a simple pit has separated longitudinally along the middle lamella and one part has glided upon the other. In places, the primary wall (*P*) is shown stained deeply with haematoxylin and at *O* it has separated along the middle lamella.

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* The presence of highly silicified longitudinal tracts in the secondary layer of wood fibers of *Tecoma* has been demonstrated by the writer and will be published in a subsequent paper on fiber skeletons.

pits may be split in a manner similar to that observed by Neeff in growing fibers of wood ('14, p. 503) and bast ('14, p. 510).

The treatment by which the middle lamella was softened is as follows: thin sections (3 μ thick) of the wood of *Dracaena* were placed for twenty-four hours in a 25 per cent alcoholic solution of hydrochloric acid, then for twelve hours in 20 per cent ammonia. After such treatment the primary wall and particularly the middle lamella were etched and softened to a degree that permitted cells to glide upon one another, the closing membranes of pits were divided along the middle lamella, so that one part moved upon another. By this treatment with acid alcohol, according to Mangin (Haas and Hill, '13, p. 127), the combined pectin is freed from its bases (e.g. calcium) and is then dissolved by the ammonia. It is of interest to note that an effect similar to that obtained by hydrochloric acid and ammonia (FIG. 2) was obtained by placing sections, 2.5 μ thick, for twenty-four hours upon wet Easter lily pollen moistened with water, the membranes, in this case, being acted upon by an enzyme. In sections of *Tecoma*, the middle lamella and primary walls were similarly etched and softened.

Not only the middle lamella and primary wall but also all other tissue elements have, with possibly a few exceptions, a characteristic refractive index; it is remarkable to what an extent the various membranes become differentiated in their refractive properties during growth. As will be shown presently, a membrane which can hardly be differentiated by reagents may be clearly defined by its refractive properties.

IV. DEFINITION OF ANATOMICAL DETAILS

Success in obtaining clear microscopic definition is in no small measure dependent upon the refraction of light by the mounting medium. It is of the utmost importance that the index of refraction of the tissue should bear a definite relation to that of the medium in which it is mounted.

1. Refraction of mounting media

When the refractive properties of a tissue are known, definite refractive effects may be obtained by selecting media having the necessary indices of refraction. The following list (TABLE II) includes some of the refractive media most useful for this purpose:

TABLE II

Refraction of media

	Index of refraction
1. Water	1.336
2. Alcohol	1.37
3. Fluorite	1.433
4. Glycerine jelly	1.44
5. Glycerine	1.46–1.47
6. Castor oil	1.49
7. Xylol	1.50
8. Oil of red cedar (*Juniperus procera*)	1.512
9. Crown glass	1.518
10. Dammar; Canada balsam	1.52
11. Clove oil	1.535
12. Quartz	1.544
13. Styrax	1.58
14. McLean's solution; flint glass	1.621
15. Balsam of Tolu	1.628
16. Naphthalene α monobromated	1.65
17. Iceland spar (calcium carbonate) (Hastings '88)	1.658 and 1.48
18. Tolu dissolved in naphthalene α monobromated	1.72
19. Quinidine	1.8?

A few comments on some of the various media listed may be made:

Water; alcohol.—These media have an index of refraction far below (a difference of about -0.20) that of the average for plant tissues. If the section is thin, these media have no superior for the definition of minute details in tissues which shrink on dehydration. But, on the other hand, a section of ordinary thickness (20–70 μ) lacks transparency in these media and the outlines are confused. In such sections, greater transparency and therefore clearer definition may be obtained by mounting in some medium such as xylol, whose index of refraction differs but little (about 0.02) from that of the tissue.

Glycerine; glycerine jelly.—Unstained sections of stem tissues, from which air has been removed by standing in alcohol, mounted in glycerine and permanently cemented with Brunswick black or gold size, require little time to prepare and are frequently superior to stained sections for observing minute details such as the pitting of fibers or vessels, as well as the broader details of outline. Glycerine jelly is particularly to be recommended for unstained sections of woody tissues.

Dammar; Canada balsam.—It is unfortunate that the refractive index of both dammar and Canada balsam approach too closely those of the stem tissues to allow their use for mounting unstained sections of any of the hygroscopic tissues of the stem. Details in some of the soft tissues of the bark, as will be shown presently, are also obscured through shrinkage during the process of dehydration. These media have a refractive index about .01 below the average for dehydrated plant tissues and, for general purposes, are fully deserving of the esteem in which they are held as media for mounting stained sections.

McLean's solution.—This solution, suggested by McLean ('14), is prepared by dissolving in a saturated aqueous solution of potassium iodide as much mercury biniodide as it will take up. This solution has a high index of refraction (1.6210 for solution of full strength), equal to that of flint glass and far exceeding that of any of the tissues examined; it is readily diluted with water, the index of refraction of which is lower than that of any constituent of plant tissue. This liquid may therefore be regarded as one of the best for preparing a series of aqueous dilutions for measuring refraction in tissues, and the principle involved in diluting with water is the same as that earlier given and adopted in mixing the oils of a refraction series. By such a series of liquids a wide range in refraction (1.33–1.62) may be covered, and if successive dilutions were made to differ from each other by $n = .005$, there would be 59 liquids in this series. As before mentioned, tissues imbibe aqueous solutions more readily than the oils, causing wider deviations in refraction, but the mean refractions of tissues determined by their use were the same as those determined by oils. McLean's solution also has high dispersion or, in other words, a wide difference between the index of refraction for light of long wave-length (red) and light of short wave-length (blue).

As a medium for mounting soft tissues, this solution possesses the advantage of not requiring dehydration, so that such tissues may be mounted directly without danger of shrinkage. But it has the serious disadvantage of causing wide deviation of refraction in certain tissues, such as collenchyma, as a result of which the outlines may become quite invisible.

Tolu.—This oleo-resin (balsam), obtained from the bark of a leguminous tree, *Myroxylon toluifera* H. B. K., of South America, brings out satisfactorily the pitting and other details of unstained macerated preparations.

Naphthalene α monobromated.—If sections are thin, naphthalene α monobromated defines clearly all tissues of the stem. Where dehydration can be accomplished without too great shrinkage of the tissue, details such as the pits in hypodermal collenchyma appear very distinctly. This oil is also one of the best for use in measuring refraction of tissues by the microscopic method.

Quinidine.—This is perhaps the most satisfactory medium of all for the definition of minute details. Also, all tissues of the bark appear clear and well defined except for details affected by shrinkage or high temperature. In using this organic substance, it is liquified on the slide at a temperature above that of boiling water. Professor Hastings states that quinidine crystallizes or spoils with time and, further, that highly refractive media, such as quinidine, tolu, and styrax, define with great clearness the minute details of diatom cysts.

2. PITS AND MIDDLE LAMELLA IN COLLENCHYMA

The part played by refraction in bringing out details may be demonstrated by comparing cross-sections of the collenchyma of some woody stem, such as *Pyrus communis* L. or *Aucuba japonica* Thunbg., mounted in Canada balsam with sections mounted in other media. In balsam mounts the prominent system of pits connecting the cells is usually obscured (FIG. 3, *A*), whether stained or not, and it is worthy of note, in this connection, that the presence of such pits in the collenchyma of woody stems, though a characteristic feature of this tissue in nearly all dicotyledonous trees and in many other woody plants, has seldom been noted in anatomical drawings or descriptions, even in such standard texts as that of Moeller ('82), although it can be readily seen when in sections which have been mounted in suitable media (FIG. 3, *B*). Thus, this pitting system becomes very clear in naphthalene α monobromated, invisible or nearly so in potassium iodide-mercury biniodide, and visible again in glycerine (FIG. 3 *B*) or water. The middle lamella, however, is not clearly defined in any of these media; but if the section is mounted in a medium whose index of refraction (about 1.526) approaches sufficiently close to that of the inner and outer thickenings (*I* and *O*, FIG. 3), then the middle lamella, whose index of refraction is higher (+) than this, stands out plainly (as indicated in FIG. 3, *C*) and is the only portion of the wall which can be seen even under the most favorable conditions of illumination. In alcohol and quinidine, dehydration may cause shrinkage of the tissues to such an extent that not as good results are obtained as would be expected from the refractive properties of these media. Glycerine, on the other hand, causes a slight swelling of the walls, but this is of advantage, in this case, in bringing out the details sought.

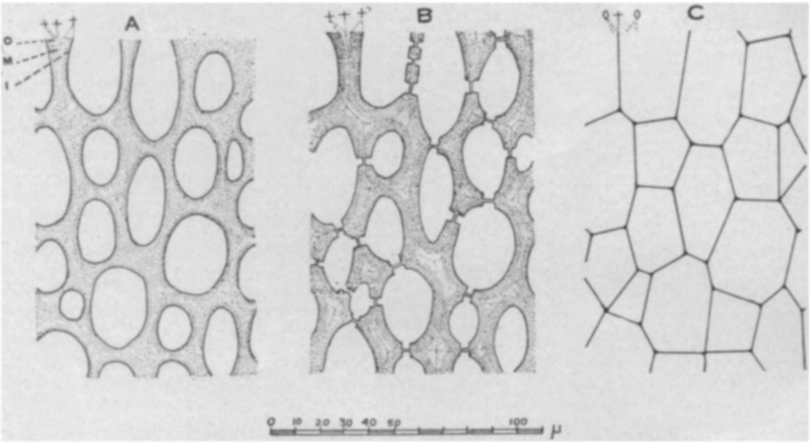

FIG. 3. Appearance of a single section of collenchyma of *Pyrus communis* L. mounted in three different refractive media. *A*, in Canada balsam, $n = 1.52$; there is little or no definition of minute detail. *B*, the same section as *A*, but mounted in glycerine, $n = 1.46$; the pits are clearly defined. *C*, again the same section, but mounted in a mixture of castor oil, 2 volumes and clove oil 8 volumes, $n = 1.526$; in a medium of this refraction, the middle lamella (*M*) is clearly defined, but the other membranes (*I* and *O*) are invisible. The tube is slightly raised from sharp focus; the inner thickening (*I*) of the collenchyma walls is less refractive than the outer thickening (*O*) and appears less brightly illuminated in *A* and *B*.

3. PERFORATIONS IN CLOSING MEMBRANE OF PITS

The minute perforations through the closing membranes of pits, as in case of the bordered pits of conifers, can be readily demonstrated in the following manner: place a few drops of a dilute solution of xylol dammar (mounting consistency diluted about twenty times) on the transverse face of the dry woody tissue to be tested. Section immediately to 10 μ and mount in glycerine. The perforations (FIG. 4) each with a small globule of dammar in or attached to it, are then easily visible in the glycerine medium. The cover glass may be cemented with gold size, after the manner of glycerine permanent mounts, and the preparation will keep for at least several weeks, often improving on standing a few days; but the slide should always be kept face upward.

In mounting tissues in highly refractive media, care should be taken to press the cover slip down tightly against the tissue, to exclude any excess liquid from above the section. With quinidine, for example, an effect of chromatic aberration may be produced by a film of the liquid between the lignified membranes of the section and the cover slip.

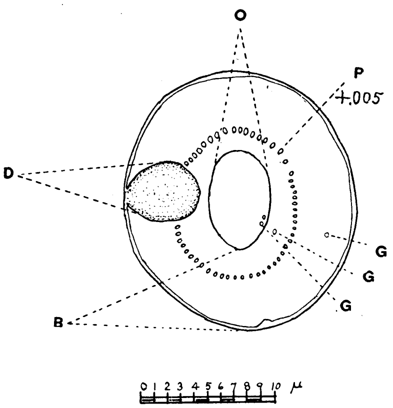

FIG. 4. Bordered pit of sugar pine, *Pinus Lambertiana*, after treatment with dammar to bring out perforations; *D*, a large globule of dammar floating partially free in the glycerine within the border (*B*); *P*, perforations through the closing membrane, arranged in a circle at the edge of the torus and visible through the transparent border; *G*, globules of dammar detached from perforation at edge of torus and floating free in the glycerine; *O*, orifice.

V. SUMMARY

1. In the fully developed tissues examined, the refractive index of the material of which any given cell-membrane or cell-content is composed is fairly constant with uniform conditions of temperature; but during the growth of a tissue the refraction of its constituent materials may change enormously. Such changes in refraction are evidently associated with corresponding changes in the chemical composition.

2. The indices of refraction of the various membranes and other elements, of which mature plant tissues are composed, differ greatly from one another, and a given tissue complex will

rarely if ever have a single refractive index. A fair average, however, would be about 1.53, or .01 above that of Canada balsam.

3. Most tissues readily absorb water and other refractive liquids, in differing amount according to the hygroscopic qualities of the tissue. This causes the refraction of the mass (tissue substance + imbibition liquid) to deviate correspondingly from that of the tissue substance. The refractive index of the membrane mass is highly sensitive to physical changes of this kind.

4. The laws of refraction have their special application to the investigation of both the anatomical characters and the physical and chemical properties of plant tissues.

5. With average sections, clearer definition is obtained when the index of refraction of the medium is below, rather than above, that of the tissue substance. A difference of at least .05 between tissue mass and the medium in which it is mounted is desirable to obtain clear definition of small perforations and similar minute details, and highly refractive media such as styrax, tolu, and quinidine bring out such details with the greatest possible clearness; in moderately thin sections, a difference of .005 is sufficient for the definition of general outlines, and may give clearer definition for general features than a greater difference in refraction.

6. In selecting a medium, to obtain a given difference in refraction between it and the tissue mass, due allowance must be made for the deviation in refraction brought about by the imbibition of liquid by the tissue.

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